8.5.4 Poxviruses 764

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764 section 8 Infectious diseases 8.5.4 Poxviruses Geoffrey L. Smith ESSENTIALS Poxviruses are large, complex DNA viruses that have played sev- eral seminal roles in medicine and biological science. Cowpox virus was introduced by Jenner as the first human vaccine in 1796; widespread vaccination with vaccinia virus led to the global eradi- cation of smallpox in 1977, the only human disease to have been eradicated. Smallpox—is caused by variola virus, the most infamous poxvirus. A systemic infection, spread by the respiratory route, with character- istic skin blisters that had a centrifugal distribution on the body and, with variola major, produced mortality rates of 30–40% in unvaccin- ated populations. Other poxviruses—molluscum contagiosum virus is the only other poxvirus that infects only humans, causing benign skin tumours that may be single or multiple, typically persisting for months be- fore undergoing spontaneous regression (see Chapter 8.5.28). Several other poxviruses may cause zoonotic infections in hu- mans, including cowpox virus, vaccinia virus, monkeypox virus, orf virus, psuedocowpox virus, tanapox virus, and Yaba monkey tumour virus. The development of vaccinia virus as an expression vector pion- eered the concept of using genetically engineered viruses as live vac- cines. Vaccinia virus is also being developed as an oncolytic agent. Poxviruses remain excellent models for studying virus-host inter- actions and virus immune evasion strategies. Introduction Poxviruses are large DNA viruses that replicate in the cell cyto- plasm. The most infamous was variola virus, which caused smallpox, a disease responsible for devastating epidemics with up to 40% mortality and that influenced human history. Smallpox was eradicated (in 1977) by immunoprophylaxis with vaccinia virus, a related orthopoxvirus. Since then, poxvirus infections in humans have been restricted to molluscum contagiosum (Chapter 8.5.28) and rare zoonoses caused by monkeypox, cowpox, orf (Chapter 8.5.27), pseudocowpox, tanapox, and Yaba monkey tumour viruses. Classification The Poxviridae is divided into the Entomopoxvirinae and Chordopoxvirinae subfamilies whose members infect insects and chordates, respectively. The Chordopoxvirinae is subdivided into ten genera and additional unassigned viruses (Table 8.5.4.1). Viruses within different genera are antigenically distinct, while those within a genus are cross-reactive and cross-protective. Orthopoxviruses have been the most important for humans (Table 8.5.4.1), and four of the nine poxviruses that infect humans are orthopoxviruses: cowpox, variola, monkeypox, and vaccinia viruses. Different orthopoxviruses are distinguishable by their biological properties, such as pock type and ceiling temperature on the chorioallantoic membrane, or by the restriction pattern of genomic DNA and the genome sequences that have enabled de- velopment of species-specific polymerase chain reaction detection methods. Vaccinia virus has no known natural animal reservoir and its origin remains a mystery. It caused human disease only as a rare complication after vaccination against smallpox. Cowpox and monkeypox viruses were named after the species from which they were

isolated, but the natural reservoir of each virus is rodents. Infections in cows or monkeys, like the occasional transmission to humans, are zoonoses. Human monkeypox virus infections are often caused by handling or consumption of infected ‘bush meat’. In 2003, there was an outbreak of monkeypox in the United States of America following the importation of Gambian rodents carrying the virus and, in 2018, a small outbreak occurred in the UK, having been imported from Nigeria. Cowpox, monkeypox, and vaccinia Table 8.5.4.1 Poxvirus classification Subfamily Genus Species Entomopoxvirinae Chordopoxvirinae Orthopoxvirus a,bVaccinia virus bVariola virus bMonkeypox virus bCowpox virus Ectromelia virus Camelpox virus Taterapoxvirus Raccoonpox virus Skunkpox virus Volepox virus Avipoxvirus aFowlpox virus Canarypox virus Capripoxvirus aSheepox virus Goatpox virus Lumpy skin disease virus Cervidpoxvirus aMule deerpox virus Crocodylidpox virus aNile crocodilepox virus Leporipoxvirus aMyxoma virus Rabbit fibroma virus Molluscipoxvirus a,bMolluscum contagiosum virus Parapoxvirus b*Orf virus bPseudocowpox virus Suipoxvirus aSwinepox virus Yatapoxvirus bTanapox virus bYaba monkey tumour virus Unassigned Squirrelpox virus a denotes the prototype for each genus. b denotes those viruses that infect humans.

8.5.4 Poxviruses 765 viruses have a broad host range, while variola virus infected only humans and the lack of an animal reservoir aided the smallpox eradication campaign. Camelpox virus is another orthopoxvirus and, like variola virus, is restricted to a single host species in which it can cause serious disease. Poxvirus biology Poxviruses replicate in the cytoplasm, encode enzymes for tran- scription and DNA replication, and have large, complex vir- ions (Fig. 8.5.4.1) and double stranded DNA genomes of 134 to 360 kbp. Vaccinia virus is the most intensively studied poxvirus. It encodes about 200 genes (the exact number varying with the strain of virus) of four classes (early 1, early 2, intermediate, and late) that are expressed in a strictly regulated manner. Transcription of each class is dependent upon the prior expression of the previous class. Virus morphogenesis is complex (Fig. 8.5.4.2a) and produces two forms of infectious virion: intracellular mature virus and extracellular enveloped virus. Some authors refer to these virions as mature virus and extracellular virus, respectively. Intracellular mature virus particles remain within the cell until cell lysis and form most of the progeny, whereas extracellular enveloped virus is released by exocytosis (Fig. 8.5.4.2b) before cell death and rep- resents a small fraction of total infectivity. The extracellular en- veloped virus possesses an additional lipid envelope with which several virus proteins are associated, giving it distinct immuno- logical and biological properties. An extracellular enveloped virus is necessary for efficient virus dissemination in vitro and within the infected host. Immunity to extracellular enveloped virus-specific antigens, which are highly conserved among orthopoxviruses, is required for protection against disease and the B5 protein on the surface is an important target against which neutralizing Abs are directed (Putz et al., 2005). Pathogenesis Poxvirus infections cause a local skin lesion or generalized pus- tular rash. Detailed experimental analysis of human smallpox was impossible, but generalized poxvirus infections have been studied in experimental models, namely monkeypox in monkeys, rabbitpox (a neurovirulent vaccinia virus) in rabbits, ectromelia virus in mice, and myxoma virus in European rabbits. The Fig. 8.5.4.1 Electron micrograph of material from a smallpox lesion, viewed by negative contrast, showing a clump of variola virus particles. Courtesy of the late Henry Bedson. (a) (b) Fig. 8.5.4.2 Electron micrographs showing (a) a cytoplasmic vaccinia virus factory containing maturing virus particles with stages of morphogenesis numbered 1 to 4 and (b) fully enveloped virus particles, one of which (number 2) is leaving the cell by exocytosis.

766 section 8 Infectious diseases spread of variola virus in humans was probably similar to that of ectromelia virus in mice, and is characterized by sequential phases of virus infection, replication and release, accompanied by cell necrosis. Studies with vaccinia virus showed that a fusion complex on the surface of intracellular mature virus particles consisting of more than 10 virus proteins is essential for virus entry into cells. Both intracel- lular mature virus and extracellular enveloped virus (after it has shed its outer membrane) can fuse with the host cell membrane at either the cell surface or within acidified vesicles, but all routes require the intracellular mature virus fusion complex. Virus enters through skin abrasions (ectromelia and cowpox) or inhalation of airborne virus and establishes a respiratory infection (ectromelia, rabbitpox, and variola). In smallpox, the respiratory route was the most important and sometimes the only possible route of transmission from index cases to contacts; also patients became infectious only after enanthem developed. A respiratory infection was established in the epithelial cells of the alveoli and small bronchioles. Here, alveolar macrophages became infected and transmitted the virus via lymphatics to the local lymph node, where further virus replication occurred. Virus released into the blood (primary viraemia) was mostly cell-associated and spread to other organs of the reticuloendothelial system, notably the liver, spleen, and lymph nodes. Extensive replication here released larger amounts of virus into the blood (secondary viraemia) enabling the virus to infect other organs such as the kidneys, lungs, and intestines and to reach the skin and produce the skin lesions with the characteristic centrifugal distribution (Figs. 8.5.4.3–8.5.4.5). Lesions started with a papule that became pustular and then crusted. After 2–3 weeks the scab was shed, leaving a scar. The incubation period of smallpox was approximately 12 days. Symptoms included headache, fever, mal- aise, vomiting, and, in severe cases, prostration, toxaemia, and hypo- tension. Delayed onset of the skin eruptions usually correlated with a grave prognosis. Haemorrhagic or flat confluent-type smallpox had very high mortality rates. The outcome of infection depended upon the age and physio- logical and immunological status of the patient and the strain of virus. Variola major was more virulent and produced fatality rates in unvaccinated patients of between 5 and 40%, while the milder variola minor, called alastrim in the Americas, caused only 0.1–2% mortality. Morphologically, the viruses were indistinguishable and vaccination with vaccinia virus was equally effective against both. However, alastrim virus was consistently more thermolabile and had a lower ceiling temperature of 37.5°C compared to 38.5°C for variola major, 39°C for monkeypox, 40°C for cowpox, and 41°C for vaccinia virus. The genomes of nearly 50 variola virus strains isolated from different places in the world at different times have been sequenced and compared, allowing the spread and evolution of variola virus in humans to be analysed. Comparisons of variola major and minor virus strains showed the genomes are very closely related, but there are too many minor differences to provide an understanding of why these viruses produced such different mor- tality rates in humans. Very young and old patients were most susceptible to smallpox and those aged 5–20 years most resistant. Pregnancy and immunological Fig. 8.5.4.3 Smallpox in a 9-month-old boy in Pakistan, photographed on the eighth day of the rash. Courtesy of the World Health Organization. (a) (b) Fig. 8.5.4.4 Ethiopian patient, in 1968, showing classical centrifugal distribution of lesions with fewer on trunk (a) than on face (b). Copyright D. A. Warrell.

 deficiency, particularly in cell-mediated immunity, increased the se- verity of infection. Pregnant women were more likely than any other group to develop haemorrhagic-type smallpox, which was usually fatal. The greater importance of cell-mediated immunity rather than antibody in recovery from poxvirus infections was illustrated in sev- eral ways. Firstly, in children with severe defects in cell-mediated immunity there was a progressive and uncontrolled virus

replication from the vaccination site that was usually fatal. In contrast, defects in antibody production were usually tolerated if the cell-mediated immune response was normal. Secondly, passive administration of antivaccinia virus serum had little effect on mice infected with ectromelia virus, whereas prior infection with vaccinia virus was protective. Thirdly, in mice infected with ectromelia virus, the ef- fective mechanisms that combated infection in the liver and spleen were operative by 4–6 days postinfection and coincided with the maximum levels of cytolytic T cells, but preceded the development of systemic antibody. The eradication of smallpox Early attempts to control smallpox relied upon variolation or in- oculation, in which material isolated from a mild case of smallpox was administered by sniffing or scratching. This was replaced by vaccination in 1798 after Jenner noticed that milkmaids, who often acquired cowpox infections on their hands from the teats of cows, were protected from smallpox. Jenner infected a boy (James Phipps) with poxvirus material (probably cowpox) derived from a cow via a milkmaid (Sarah Nelmes) and challenged him subsequently with smallpox. Protection was achieved and, due to the efficacy and greater safety of this procedure, it replaced variolation rapidly. Sometime between 1798 and the 20th century, vaccinia virus re- placed cowpox virus as the smallpox vaccine. In 1959, the World Health Assembly (WHA) adopted a recommendation to achieve the global eradication of smallpox. With fresh funding and a plentiful supply of potent freeze-dried vaccine this goal was achieved in 1977. Two years later, the World Health Organization (WHO) certified that eradication was complete. This triumph of preventive medicine justifies the saying ‘prevention is better than cure’, but also demon- strates that prevention is best achieved by eradication. Posteradication issues Following the eradication of smallpox the WHO sought to centralize all known stocks of variola virus and these stocks are now held in maximum security laboratories (Biosafey Level 4) in the United States and the Russian Federation. These facilities are inspected regu- larly by the WHO and any work with live virus requires prior per- mission from the WHO. In 1996 the WHA passed a resolution that these stocks of virus should be destroyed. Since then temporary re- tention of these viruses has been authorized to enable research that is essential for public health benefit to be completed. This research aims to develop (1) diagnostic tests that can swiftly identify cases of smallpox and distinguish this from infections caused by other vir- uses; (2) drugs that can treat smallpox; and (3) a safer vaccine that can be tolerated by those for whom the current vaccine is contra-indicated. Good progress towards these objectives has been achieved. Poxvirus genomes The DNA sequence of more than 100 orthopoxvirus genomes has been determined (see http://www.poxvirus.org), including about 50 strains of variola virus and at least one strain of most orthopoxviruses. The central region (about 100 kb) of these genomes is very highly conserved and 89 of the genes within this region are present in every sequenced chordopoxvirus. These genes probably represent the core genome of an ancestral poxvirus from which the current poxviruses evolved. During their evolution poxviruses acquired additional genes that became located in the more variable terminal regions of the genome and these give each virus its characteristic host range, virulence, and tropism. These genes vary in number and type be- tween poxviruses, and encode nonessential proteins that affect virus virulence, host range, and immune modulation.
A surprising feature of some orthopoxviruses is the fragmentation of several genes that are intact in other viruses, indicating that orthopoxvirus evolution has involved both gain and loss of gene function. The retention of these nonfunctional genes by some viruses, such as variola, suggests that they became nonfunctional in the relatively recent evolutionary past, and perhaps that variola virus is a ‘recent’ human pathogen that never became fully adapted to humans. In 2016, the sequence of a variola virus genome was obtained by sequencing DNA from a mum- mified child from Lithuania who died in about 1650. This sequence is evolutionarily basal to all other variola

genomes, contains the same pattern of gene degradation found in 20th century variola viruses, and suggests that the genetic diversification of variola virus in man was more recent that thought hitherto (Duggan et al., 2016). Fig. 8.5.4.5 Moderately severe monkeypox in a girl of 7 years from Équateur Province, Democratic Republic of the Congo. Courtesy of the World Health Organization.

768 section 8 Infectious diseases Recreation of poxviruses by synthetic biology Since 2012 it has been possible to recreate an infectious poxvirus from cloned DNA (Domi et al., 2002). This was achieved by trans- fecting the cloned virus genome into cells that are infected with a helper poxvirus. Furthermore, advances in synthesis of DNA have enabled recreation of an entire poxvirus genome from simple chem- icals and from this genome to recreate infectious virus. Given that the genome sequence of variola virus is in the public domain, it is therefore possible to recreate an infectious variola virus from simple chemicals. Although such activity is prohibited absolutely by the WHO, the fact that this is possible means that the potential for variola virus and smallpox to reappear can never be completely eliminated. Poxvirus expression vectors Vaccinia virus recombinants expressing foreign genes were developed in 1982 and have become a widely used laboratory tool; they are also being engineered as live vaccines for infectious disease and cancer. Infection with the recombinant virus allows expression and simultan- eous delivery of the foreign antigen to the immune system. Moreover, the large capacity of vaccinia virus allows expression of multiple for- eign genes from a single virus so creating polyvalent vaccines. Safer vaccinia virus strains that do not cause vaccination complications (ec- zema vaccinatum, generalized vaccinia, progressive vaccinia, enceph- alopathy (<2 years), or encephalitis (>2 years)) are being created by genetic engineering. An alternative strategy is to use poxviruses that establish only abortive infections in human cells, such as modified vac- cinia Ankara or the avipoxviruses fowlpox virus and canarypox virus. Human monkeypox Monkeypox was discovered in captive primates in 1958, but in 1970 was isolated in the tropical rainforests of West and Central Africa from humans who had suffered generalized poxvirus rashes visibly very similar to smallpox. The virus is quite distinct from variola in biological properties such as pock morphology, ceiling temperature, and lesion morphology on rabbit skin, and its genome sequence. Moreover, although monkeypox virus produced a very similar dis- ease to smallpox in humans, person-to-person transmission was inefficient. Thus, human monkeypox virus infections are single or multiple sporadic cases restricted to dense tropical rainforests in Central and West Africa. Clinically, human monkeypox closely resembles ordinary, discrete-type smallpox except that there is a pronounced lymph node enlargement (Fig. 8.5.4.5). Two clades of monkeypox virus have been identified (from Central or West Africa) that differ in their virulence in humans. The Central African strains gave mortality rates in unvaccinated children (<8 years old) between 1970 and 1986 of 11.2%. In contrast, West African strains, such as the one that caused an epidemic in the United States of America in 2003, are milder and no mortalities were reported. Prevention and treatment In endemic parts of Africa, or in the face of new epizootics, use of current smallpox vaccine has been discussed, but the prevalence of HIV/AIDS in some areas would restrict widespread, safe use of this approach. Cidofovir treatment cured monkeys infected with monkeypox virus. Cowpox virus and pseudocowpox virus Cowpox virus has a broad host range including cattle, humans, large felines, and even elephants, but it is not enzootic in cattle and its nat- ural hosts are rodents. It is distinguishable from vaccinia virus by the pock type, ceiling temperature, genome size and sequence, and the production of cytoplasmic type A inclusion bodies. Pseudocowpox is enzootic in cattle, unlike cowpox. Historically, pseudocowpox virus was important because it was sometimes used mistakenly for vaccination and, being a parapoxvirus, was ineffective in preventing smallpox. Its misuse compromised Jenner’s correct assertion that cowpox virus was an effective smallpox

vaccine. In humans, cowpox virus produces an acutely inflamed, local lesion, similar to a primary smallpox vaccination. There is usually fever, enlargement of the local lymph nodes, and pain. Unlike vac- cinia virus, which occasionally produced a generalized infection (Fig. 8.5.4.6), cowpox virus lesions are always local. Human lesions caused by pseudocowpox virus (milker’s nodules) are extremely rare and are less painful than those caused by cowpox. Tanapox virus and Yaba monkey tumour virus Tanapox virus and Yaba monkey tumour virus are the sole members of the Yatapoxvirus genus (another yatapoxvirus called Yaba-like Fig. 8.5.4.6 Generalized vaccinia. Courtesy of the late Dr B. E. Juel-Jensen.

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